Investigating the Cell Division Protein FtsZ and its Regulation in *Bacillus subtilis*

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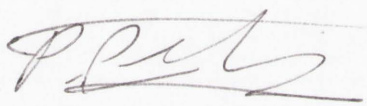
Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it, has been carried out solely by me, unless otherwise indicated. Finally, I certify that all information sources and literature used are acknowledged in the text.



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Abbreviations

$A_{\#}$	absorbance (# refers to the wavelength in nm)
A	alanine
aa	amino acid
Ab	antibody
AGRF	Australian Research Genome Facility
AP	alkaline phosphatase
ARC	Australian Research Council
ATM	atomic force microscopy
B.	*Bacillus*
β	beta
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
cm	centimeters
Cm^R	chloramphenicol resistance
CCD	charged coupled device
DAPI	4'6-diamidino-2-phenylindole
dATP	**deoxyadenosine** 5'-triphosphate
dCTP	**deoxycytidine** 5'-triphosphate
dGTP	d**eoxyguanosine** 5'-triphosphate
DIC	differential interference contrast
DOL	degree of labelling
DNA	deoxyribonucleic acid
DNA pol	DNA polymerase III holoenzyme
dpm	decays per minute
dsDNA	double stranded deoxyribonucleic acid
DTT	dithiothreitol
dTTP	**deoxythymidine** 5'-triphosphate
DVD	digital versatile disc
E.	*Escherichia*

ECT	electron cryotomography
et al.	and others
FABLS	Fluorescence Applications in Biotechnology and Life Sciences
FAD	flavin adenine dinucleotide
FFT	Fourier transform analysis
FITC	fluorescein isothiocyanate
FRAP	fluorescence recovery after photobleaching
FRET	fluorescence energy resonance transfer
fts	filamentation temperature sensitive
FWHM	full width half maximum
g	centrifugal force
g	gram(s)
GFP	green fluorescent protein
GMD	germination medium defined
hr	hour(s)
HPUra	6-(-p-hydroxyphenylazo)-uracil
$HPUra^R$	HPUra resistant
IFM	immunofluorescence microscopy
IgG	Immunoglobulin G
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kD	kilo Dalton(s)
L	litre(s)
LP	long pass
m	milli- (10^{-3})
M	moles per litre
min	minute(s)
MQW	Milli-Q purified water
MSA	mineral salts A
MTS	membrane targeting sequence
n	nano- (10^{-9})
NA	numerical aperture
N/A	not applicable

NHS	N-hydroxysuccinimide
NO	nucleoid occlusion
NS	nucleation site
OD	optical density
P	probability
P_{spac}	IPTG-inducible promoter
$P_{spac\text{-}hy}$	IPTG-hyper-inducible promoter
P_{xyl}	xylose-inducible promoter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pers. comm.	personal communication
pH	power of Hydrogen
PSF	point spread function
RNA	ribonucleic acid
RNase	ribonuclease A
ROR	round of replication
ROW	reverse osmosis purified water
rpm	revolutions per minute
S.	*Streptomyces*
S	serine
sec	second(s)
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SMC	structural maintenance of chromosome
SMM	spizizen minimal medium
spp.	species
spec	spectinomycin
STED	Stimulated emission depletion
T	thymine
TBAB	tryptose blood agar base
TBP	tributyl phosphate

TDE	2,2'-thiodiethanol
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
tet	tetracycline
TFA	trifluoroacetic acid
thy^-	thymine auxotroph
Tris	tris(hydroxymethyl)methylamine
Trp	L-Tryptophan
ts	temperature sensitive
U	units (enzyme activity)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
W	watt
w/v	weight per volume
YFP	yellow fluorescent protein
2D	2-dimensional
3D	3-dimensional
4D	4-dimensional
μ	micro- (10^{-6})
μCi	micro curie

Abstract

As with all organisms, bacterial cells divide with amazing precision. The first stage of this process is marked by the polymerisation of the essential tubulin-like FtsZ protein at midcell into a ring, the Z ring. Understanding its formation and regulation provides an insight into how the crucial event of cell division is controlled. However, despite intense investigation, these molecular mechanisms are not fully understood. One factor believed to play a role in midcell Z ring placement is the coordination between DNA replication and cell division. Previously it has been shown that when initiation of DNA replication is allowed, but DNA synthesis is inhibited by two different methods (thymine starvation or addition of HPUra), Z rings are able to form at midcell in one case, and not in the other. Both conditions block DNA synthesis at the same stage, the beginning of DNA chain elongation. In an attempt to understand these incongruous results, the possibility of the drug HPUra playing a non-replicative role, leading to displacement of the Z ring, was examined. It was found that Z ring positioning in an HPUra-resistant strain was not significantly different to that of wild type. Z rings formed at midcell in both conditions. Thus in the wild type strain, the effect of HPUra on Z ring positioning is dependant on its ability to inhibit replication. Hence the block to the elongation stage of DNA replication mediated by the addition of HPUra is capable of misplacing the Z ring, strong evidence for a link between these essential processes of DNA replication and cell division.

Ten years ago it was proposed that the Z ring forms by bidirectional growth from a midcell nucleation site. Work presented in this thesis now suggests this may not be the case. Using a modified immunofluorescence protocol it was discovered that, in addition to forming a Z ring, FtsZ forms a helical structure along the length of the cell in vegetatively growing wild type *Bacillus subtilis* cells. Time-lapse experiments in live cells using an inducible FtsZ-YFP fusion, showed that the helical FtsZ structure is highly dynamic and undergoes cell cycle-regulated changes in localization. The monitoring of a complete cell cycle revealed the early appearance of a pole-to-pole FtsZ helix, a subsequent short helix spatially restricted to midcell, and finally this redistributed to produce a sharp midcell Z ring. These observations led to the proposal of a novel assembly mechanism for Z ring formation

involving a cell-cycle mediated multi-step remodelling of FtsZ polymers. This was the first report of an FtsZ helix in *B. subtilis* during vegetative growth.

The new model for Z ring formation predicts that in order for the cell to assemble a Z ring, FtsZ must go through long helix-to-short helix-to-Z ring polymerisation changes. How is the Min system, a known negative regulator of FtsZ responsible for inhibiting aberrant Z ring assembly at the cell poles, involved in regulating these FtsZ polymerisation transitions? To address this, FtsZ polymer remodelling was examined whilst modifying the effect of the Min system. Time-lapse studies of a strain carrying a deletion of the *minCD* genes showed FtsZ polymerising at the poles in the same fashion as wild type; that is going through a short helical intermediate prior to Z ring formation. This indicated that the helical form of FtsZ is in fact a true intermediate, required for Z rings to form even at non-midcell locations. A *minCD* over-expression strain showed a marked decrease in Z ring formation and time-lapse imaging was conducted to assess at which transitional stage FtsZ assembly was affected. Interestingly, it was found that both the long and short helical polymerisations of FtsZ can actually form as wild type in the over-expression experiments. The excess of MinCD in the cell appeared to be able to severely impair division by hampering and prolonging the transition of the short midcell helix to a ring. It is proposed that this is mediated by the inhibition of lateral interactions of FtsZ protofilaments. Indeed a model is put forth emphasizing the importance of lateral interactions in the helix-to-ring remodelling, and thus in stable Z ring formation.

To examine the *in vivo* FtsZ helix with higher resolution, the advanced microscopic techniques of 4Pi and STED imaging were employed. Using alternative methods has the advantages of confirming the helical structure and extracts further information, for example is the helix continuous? STED microscopy breaks the diffraction barrier and lateral resolution is increased to ~100 nm, ~2.5 times that of normal confocal microscopy. Using STED, FtsZ localization showed a distinct periodicity, consistent with a helical conformation. Additionally FtsZ staining was revealed to be extremely punctate and discontinuous, suggesting that the helical structure of FtsZ may depend on a cellular track. Visualising cells and their sub-cellular structure in ever increasing detail ensures novel insights into the regulation of Z ring assembly in bacteria.